REMARKS

Claims 1, 3-5, 11, 16-20 and 22-27 are presently pending. Of these, Claims 16-20, 22 and 23 are withdrawn from consideration. The limitation of canceled Claim 2 is incorporated into Claim 1. Claims 6 and 7 are canceled without prejudice. No new matter has been added herewith. The following addresses the substance of the Office Action.

Enablement

Claims 1-7, 11 and 24-27 were rejected under 35 U.S.C. § 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. In particular, the Examiner indicated that the claim should specify the buffer condition and pH that selectively elute fibrinogen and the at least one other protein separately from the immobilized metal ion affinity chromatography (IMAC) matrix.

The Applicants have amended Claim 1 to recite a method for the specific separation and purification of fibrinogen and plasminogen using an IMAC matrix. Based on the teaching of the specification as a whole and the common general knowledge, the skilled person, if set the task of reproducing the method according to presently amended claim 1, would understand which conditions affect the binding of fibrinogen and plasminogen and that it would be a matter of routine experimentation to optimize these conditions to ensure that the relevant binding affinities are obtained. In this regard, page 6, lines 10-37 of the present application as filed disclose several of the variables that affect the binding affinity of fibrinogen and plasminogen to the matrix. Notably, page 6, lines 26 to 34 discuss the various factors that may be adjusted to ensure that plasminogen is selectively eluted from a column which has both fibrinogen and plasminogen bound to it. Such conditions include the pH and ionic strength of the solution, and the concentration of various competitive chelators. By extension, it can be inferred that these conditions, although specified as affecting how easily the proteins are eluted from the column, would also effect whether the proteins initially bind to the column. Furthermore, the numerous examples contained in the application as filed provide an extensive reference for the skilled person as to how these various factors affect the binding affinity of fibrinogen and plasminogen.

Accordingly, when set the task of optimizing the conditions for binding fibrinogen and plasminogen to an IMAC matrix, the skilled person, guided by the examples in the application as filed and using the common general knowledge, would easily be able to select experimental

conditions to ensure that the necessary binding affinities for fibrinogen and plasminogen are obtained. Limiting the method of the invention to include variables such as the buffer condition and pH that selectively elute fibrinogen and plasminogen separately from the IMAC matrix would be unduly limiting, since the value of any given parameter would depend on each of the other parameters in the system. Furthermore, each of the parameters is readily derivable by the skilled person as a matter of routine experimentation.

In view of the amendment to Claim 1 and the preceding remarks, the Applicants respectfully request that the rejection be withdrawn.

Anticipation

U.S. Patent No. 5,169,936

Claim 1 was rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,169,936. US 5,169,936 discloses the use of IMAC for the purification of proteins from solutions containing contaminants. In particular, the examples of US 5,169,936 disclose the use of IMAC for the purification of rsT4. The final paragraph of the reference indicates that the process disclosed therein may be used to purify other proteins, such as *inter alia* human fibrinogen (see column 10, line 38). However, this disclosure is merely speculative, as fibrinogen is only one in a long list of other possible proteins which may be used in the process of US 5,169,936. Furthermore, US 5,169,936 provides no indication that IMAC may be used in the separation of fibrinogen from other proteins, much less the separation of fibrinogen and plasminogen. Indeed, there is no mention of plasminogen, anywhere in US 5,169,936. Accordingly, amended Claim 1 is novel with respect to US 5,169,936.

Hari et al.

Claim 1 was also rejected under 35 U.S.C. § 102(b) as being anticipated by Hari et al. (2000 *J Biomed Mater Res* **50**:110-112). Hari et al. discloses the use of IMAC for the selective absorption of human IgG from a mixture of albumin, γ-globulin, fibrinogen and IgG. However, Hari et al. does not explicitly disclose the presence of plasminogen in the process disclosed therein. Therefore, presently amended Claim 1 is novel over Hari et al.

Obviousness

Claims 1-7, 11 and 24-26 were rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 95/25748 in view of Hari et al. (*supra*) and Chaga et al. (2001 *J Biochem Biophys Methods* **49**:313-334).

WO 95/25748 discloses a method for the co-purification of fibrinogen and factor XIII which comprise the steps of loading a solution comprising fibrinogen, factor XIII and plasminogen onto a cation affinity column such as a lysine affinity column (e.g., lysine Seraphase 4B) under conditions such that the plasminogen is removed by absorbing to the lysine seraphase column. Figure 1 of WO 95/25748 succinctly shows the complexity of the process disclosed therein, which requires multiple steps to produce the fibrinogen. For example, page 7, lines 10 to 23 indicate the need to add a protease inhibitor to prevent any interaction between the fibrinogen and any thrombin that may be present, which protease inhibitor must subsequently be removed during the process.

The Examiner stated that the present invention differs from the teachings of WO 95/25748 only in that the column is a metal ion affinity column instead of a lysine affinity column. However, the Examiner has overlooked a novel aspect of the claimed methods, wherein fibrinogen and/or plasminogen specifically bind to a metal ion affinity column matrix, even in the absence of a recombinantly engineered tag, such as a multiple his tag. Thus, the use of a metal ion affinity column matrix is not a mere substitution of the type of column disclosed by WO 95/25748. Binding of fibrinogen and/or plasminogen to an IMAC matrix is an intrinsic property of these proteins that was previously not appreciated by the prior art. As such, the starting material for the methods of the invention may be any fibrinogen-containing solution, including human or animal plasma or a plasma fraction, cell culture fractions from recombinant technology, fractions derived from milk from transgenic animals, etc. (*see*, the Specification as filed at page 4, lines 31-36). The unexpected ability of fibrinogen and plasminogen to specifically bind to an IMAC matrix was not known in the prior art. Nothing in the cited references would have given any reason to the skilled artisan to believe that even native fibrinogen and/or plasminogen would bind to an IMAC matrix. The unexpected results obtained by the present applicants rebut the alleged *prima facie* obviousness.

The Examiner stated that the skilled person would be led to the method of the present invention by the teaching of Hari et al. In formulating this view, the Examiner has taken the position that all of the proteins disclosed in Hari et al., namely fibrinogen, human IgG, albumin and γ-globulin, bind to the IMAC matrix. However, the relevant passage relied on by the Examiner does not state that fibrinogen binds to the IMAC matrix (see the paragraph spanning columns 1 and 2 on page 110 of Hari et al., penultimate sentence). Instead, this passage merely describes the "selective adsorption of IgG from a mixture of ....fibrinogen...," suggesting that IgG is adsorbed whilst fibrinogen is not. Furthermore, the experimental protocols provided on page 111 (as referred to by the Examiner) do not refer to fibrinogen binding in the IMAC matrix, and merely describe the experimental procedure by which the tests were performed. Moreover, the paragraph spanning columns 1 and 2 on page 112 of Hari et al. indicate that "fibrinogen adsorption is negligible". Thus, Hari et al. teaches away from the presently claimed methods by concluding that fibrinogen did not bind significantly to an IMAC matrix. Hari et al. achieved separation of IgG, albumin and γ-globulin as a result of the fact that fibrinogen did not bind to the IMAC matrix. Furthermore, Hari et al. is completely silent regarding the binding affinity of plasminogen to an IMAC column. Therefore, Hari et al. would not have led the skilled person to adapt the process disclosed in WO 95/25748 to arrive at the method of the presently claimed invention. Even if the skilled person were to investigate a method using IMAC to separate fibrinogen and plasminogen, Hari et al. would lead the skilled person to try using IMAC to bind plasminogen, since Hari et al. teaches that fibrinogen would not bind to an IMAC column. However, in practice this would not work, since as demonstrated by the Examples of the present invention the binding of plasminogen to IMAC is weaker than the binding of fibrinogen, so fibrinogen would not flow through in the unbound fraction.

The Examiner further cites Chaga et al., asserting that this document indicates that IMAC is so popular that a skilled person would adopt IMAC when looking to modify the methods disclosed in WO'748. However, the fourth paragraph on page 313 of Chaga et al. indicates that IMAC is one of the most popular methods for purification of recombinant proteins. The method of the present invention involves the purification of naturally occurring proteins in plasma, which are not recombinant. Recombinant proteins typically have a particular IMAC binding affinity "designed in" during the artificial synthesis of the structure. For example, a peptide tag

containing multiple histidines may be incorporated at the N- or C-terminus of a recombinant protein to facilitate binding to a metal column. Chaga et al. is completely silent on the use of IMAC in the separation of mixtures of naturally occurring proteins such as fibrinogen. In fact, Section 5 on page 318 of Chaga et al. quotes Sulkowski (1989 *BioEssays* **10**:170-175), who called for "a need to develop chromatographic protocols providing for the resolution of proteins...," and then indicated that more than a decade later there are no simple generic protocols. The overall disclosure of Chaga et al. generally promotes and summarizes the use of IMAC, without teaching any particular methods. Absent hindsight knowledge of the present invention, there is nothing in Chaga et al. that would have led the skilled person to expect that an IMAC matrix could be used to separate fibrinogen and plasminogen. If anything, Chaga et al. appears to be calling for the development of new methods of separating proteins using IMAC. It should be noted that the present invention is based on the discovery of a method for purifying fibrinogen and plasminogen based on the unexpected property of fibrinogen and plasminogen specifically binding to an IMAC matrix. Accordingly, the use of IMAC matrices to purify recombinant proteins, as disclosed by Chaga et al., is not relevant.

Turning to the other documents cited by the Examiner, US 5,169,936 discloses the separation of one protein from an unspecified impurity. A disadvantage of US 5,169,936 is that it requires the material to be loaded onto the column in the same weak ligand which is later used to elute the target protein. Column 8, lines 8 to 11 of US 5,169,936 indicate that the use of a different weak ligand as the eluant decreases the efficiency of the process. The method of presently amended Claim 1 has no such requirement. Furthermore, US 5,169,936 provides no indication that IMAC may be used to effectively and efficiently separate and purify fibrinogen and plasminogen. In fact, the reference makes no mention of plasminogen at all.

Referring to Claim 11, fibrinogen produced by the method of the invention is both novel and inventive over the fibrinogen products known in the art. Using electrophoresis, the applicants have found that fibrinogen produced by the method of Claim 1 contains less degraded fibrinogen than other commercially available products. This suggests that the method of manufacture using metal chelate chromatography is not as aggressive as more conventional precipitation methods, resulting in less damage to the complex fibrinogen molecule. A less

damaged fibrinogen molecule will have greater functional activity in relation to the various reactions it undergoes (e.g., proteolytic cleavage and cross-linking).

The process of the present invention also provides fibrinogen with reduced plasminogen. Other commercial pharmaceutical products contain more plasminogen, which explains their need to include fibrinolysis inhibitors such as aprotinin or tranexamic acid. Referring to page 5, lines, lines 23-32 of the Specification as filed, it is preferred that plasminogen be removed, so that the addition of plasmin inhibitors to fibrinogen preparations may be avoided. Purified fibrinogen obtained by the presently claimed IMAC methods is distinct from known fibrinogen products, and is both novel and inventive over the cited art.

Claim 27 was rejected under 35 U.S.C. § U.S.C. 103(a) as being unpatentable over WO 95/25748 in view of Hart et al. (*supra*), Chaga et al. (supra), and WO 96/17631. However, in view of the remarks above, since the method of Claims 1 and 3 are not obvious in light of WO 95/25748 in view of Hart et al. (*supra*), Chaga et al. (supra), adding WO 96/17631 does not support a case of *prima facie* obviousness.

In summary, none of the documents, either alone or in combination, would have led the skilled person to the presently claimed invention. Accordingly, the Applicants respectfully request that the rejections under 35 U.S.C. § 103(a) be withdrawn.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

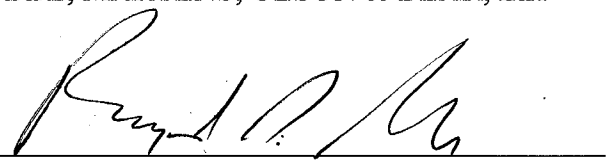
In view of Applicants' amendments to the Claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: March 16, 2009

By: 

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